EXERCADOL

Statistical Analysis Plan

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1. General information of EXERCADOL study

1.1. Background and rationale

Besides cardiotoxicity caused by chemotherapy (notably, but not only, anthracyclines), different treatments (cranial irradiation, corticosteroids) can provoke cardiometabolic impairments up to several decades after treatment in survivors of childhood/adolescent cancer compared with non-cancer controls (i.e., worsened echocardiography-determined left ventricular (LV) systolic function in the former, alongside an unhealthier lipid and adiposity profile). In addition, adolescents treated for cancer frequently engage in health-risk behaviors that can further impair cardiometabolic health such as physical inactivity and poor nutrition. Recent moderate evidence, based on randomized controlled trials (RCTs), suggest that concurrent (aerobic and strength) exercise training can improve two common cancer/treatment-related health outcomes in patients with children/adolescent cancer, muscle strength and physical function. There is, however, no RCT-based evidence for other important outcomes such as echocardiography-determined cardiac function and no study has focused solely on adolescents, reflecting a gap in the current state of knowledge.

1.2. Objective

The main aim (exercise in adolescents, EXERCADOL) is to determine the effects of a supervised concurrent exercise intervention combined with usual care, compared with usual care alone, in adolescents with cancer on echocardiography-determined LV function (primary outcome). We also assess intervention effects on several cardiometabolic and overall health-related variables (secondary outcomes).

The primary aim is to determine the effects of a supervised concurrent exercise intervention combined with usual care, compared with usual care alone, in adolescents with cancer on echocardiography-determined LV ejection fraction, LV fractional shortening and LV global longitudinal strain (primary outcomes).

Secondary aims are to:

i. investigate the effects of a supervised concurrent exercise intervention combined with usual care, compared with usual care alone, in adolescents with cancer on other echocardiography-determined LV function variables (LV mass, interventricular septum thickness, LV end-diastolic volume, LV posterior wall thickness, relative wall thickness, LV hypertrophy).
ii. investigate the effects of a supervised concurrent exercise intervention combined with usual care, compared with usual care alone, in adolescents with cancer on arterial blood pressure variables.
iii. investigate the effects of a supervised concurrent exercise intervention combined with usual care, compared with usual care alone, in adolescents with cancer on blood biomarkers (N-terminal prohormone of brain natriuretic peptide (NT-proBNP) and high-sensitivity cardiac troponin-I (cTni), cardiometabolic-related proteome, inflammation (high-sensitivity C-reactive protein, hsCRP), serum lipid profile, glucose control, blood immune phenotype and gut microbiome).

iv. investigate the effects of a supervised concurrent exercise intervention combined with usual care, compared with usual care alone, in adolescents with cancer on anthropometry (body mass index and waist-to-hip ratio).

v. investigate the effects of a supervised concurrent exercise intervention combined with usual care, compared with usual care alone, in adolescents with cancer on DXA-determined body composition (lean and fat mass, visceral fat, and bone mineral density and content).

vi. investigate the effects of a supervised concurrent exercise intervention combined with usual care, compared with usual care alone, in adolescents with cancer on energy intake (energy and substrate).

vii. investigate the effects of a supervised concurrent exercise intervention combined with usual care, compared with usual care alone, in adolescents with cancer on physical activity and sedentary behaviors.

viii. investigate the effects of a supervised concurrent exercise intervention combined with usual care, compared with usual care alone, in adolescents with cancer on cardiopulmonary exercise testing (volume of oxygen uptake and power output at both peak and at the ventilatory threshold).

ix. investigate the effects of a supervised concurrent exercise intervention combined with usual care, compared with usual care alone, in adolescents with cancer on muscle strength (dynamic muscle strength, handgrip strength and maximum inspiratory muscle strength).

x. investigate the effects of a supervised concurrent exercise intervention combined with usual care, compared with usual care alone, in adolescents with cancer on functional mobility variables.

xi. investigate the effects of a supervised concurrent exercise intervention combined with usual care, compared with usual care alone, in adolescents with cancer on psychological status (health-related quality of life and cancer-related fatigue).

xii. investigate the effects of a supervised concurrent exercise intervention combined with usual care, compared with usual care alone, in adolescents with cancer on clinical variables (survival, treatment tolerability, number and duration of viral/bacterial/fungal infections, treatment-related side effects (or 'events') and toxicities).

2. Study methods

2.1. Trial design

The EXERCADOL study is a two-arm RCT carried out during cancer treatment (i.e., during the whole neoadjuvant (for solid tumors) or intensive (leukemias) chemotherapy, expected median duration 5-6 months (expected range duration 14 to 28 weeks)). At least 136 adolescents (male/female, 12-19 years) with an extracranial malignancy are being recruited from 4 hospitals (Madrid, Spain) and are randomly allocated to an intervention or standard care (control) group. The intervention consists of supervised concurrent (aerobic+resistance) exercise sessions (thrice a week) performed primarily in the relevant hospital gymnasium or alternatively in the patients' ward (i.e., due to immunodepression) or online (for those unable to attend in person). It also includes specific respiratory muscle training (5 days/week). The two groups will receive standard treatment and health (psychological, support and educational)

counselling. More detailed information is described in ClinicalTrials.gov (NCT05539794). **Figure 1** shows the study design. The EXERCADOL research team will perform the data curation and statistical analyses.

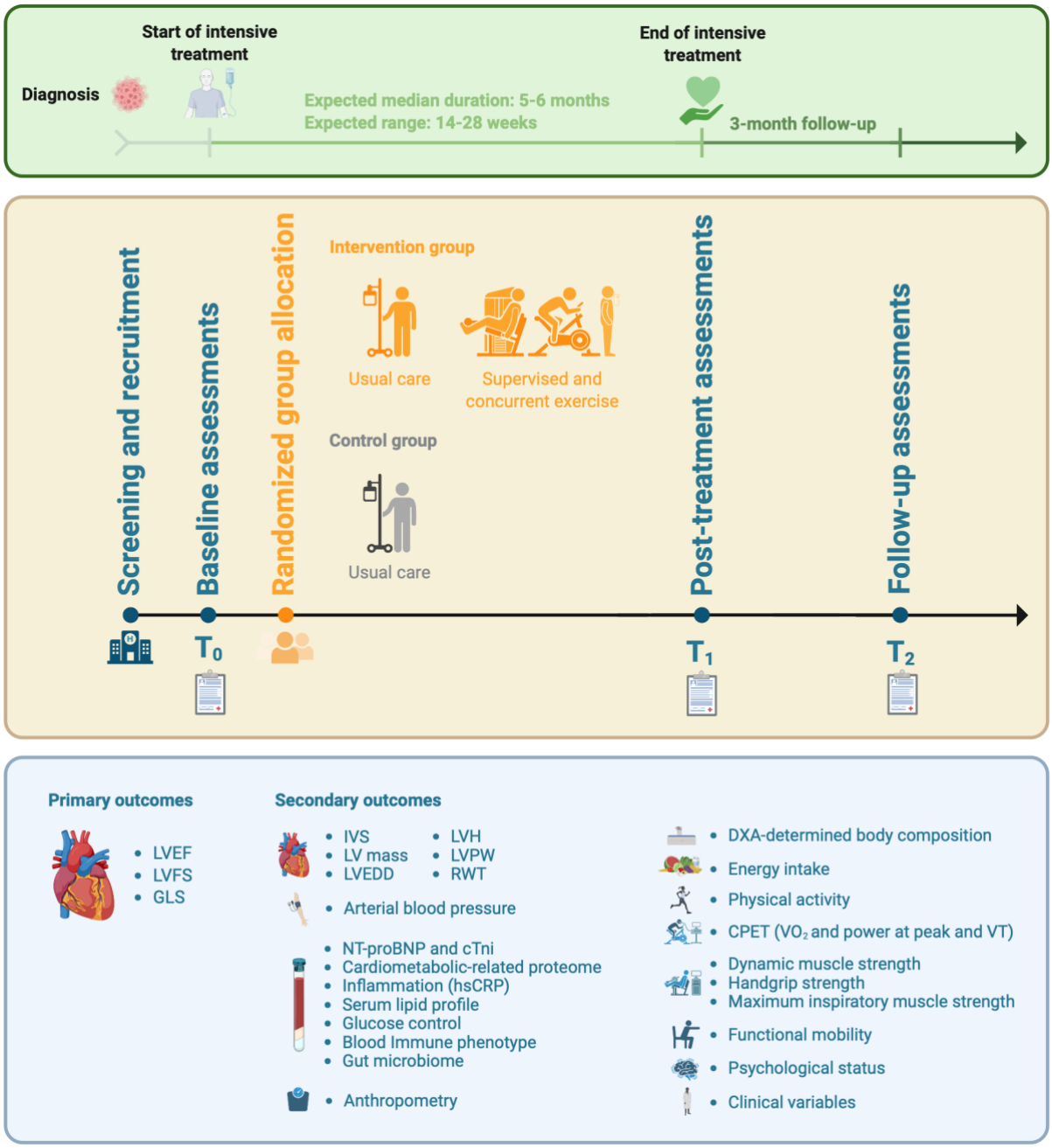

Figure 1. Overview of the EXERCADOL Study. Abbreviations: CPET, cardiopulmonary exercise testing; cTni, cardiac troponin-I; DXA, dual-energy X-ray absorptiometry; GLS, global longitudinal strain; hsCRP, high-sensitivity C-reactive protein; IVS, interventricular septum thickness; LV, left ventricular; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; LVH, left ventricular hypertrophy; LVPW, left ventricular posterior wall thickness; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; RWT, relative wall thickness; T, time; VO_{2peak}, peak volume of oxygen uptake; VT, ventilatory threshold.

2.2. Randomization

Adolescents are randomized using a parallel design with a 1:1 allocation ratio using a computer-generated random allocation sequence with a block on sex and tumor type (leukemias/solid tumors), after completing the preintervention measurements.

2.3. Sample size

Compared with controls with no history of cancer, survivors of childhood/adolescent cancer have reduced LV function (primary outcome of our study, weighted mean ± standard deviation for LVEF=67±7% *vs* 64±5%, p<0.01).[1] On the other hand, we have shown that an inhospital exercise intervention performed along the entire treatment course significantly attenuates the negative impact of cancer/treatment on children/adolescents' LVEF upon treatment termination as well as at 1-year follow-up.[2] Based on these results,[2] and assuming a mean effect size=0.67 at post-treatment in the intervention group (n=22, LVEF=69±6% (pre-treatment) and 69±6% (post-treatment)) vs controls (n=24, 69±6% and 65±6%),[2] to find a significant intervention effect with a statistical power >95% (p<.05, two-tailed) we need to recruit ≥136 participants (after accounting for a dropout rate of ~15% to prevent underpowering of two-arm RCTs).[3]

2.4. Framework

We will use a superiority hypothesis testing framework. In the main analyses, we will compare whether a supervised concurrent exercise intervention combined with usual care is superior to usual care alone.

2.5. Statistical interim analyses and stopping guidance

No pre-specified interim analyses are performed and hence, a stopping guidance will not be applicable.

2.6. Timing of the final analyses

The final analyses will be conducted after the data collection completion and processing of the primary and secondary outcomes.

2.7. Timing of outcome assessment

The primary (i.e., echocardiography-determined LV function) and secondary outcomes (i.e., other echocardiography-determined LV function variables, arterial blood pressure variables, blood biomarkers, anthropometry, DXA-determined body composition, energy intake, physical activity, cardiopulmonary exercise testing, muscle strength, functional mobility variables, psychological status and clinical variables) will be assessed at baseline (diagnosis), end of treatment, and at 3-month post-treatment (follow-up) by the same trained staff to ensure consistency and reliability. The time frame of each outcome is explained in section *'5.1 Outcome definitions'*.

3. Statistical principles

3.1. P values

To address multiplicity due to the large number of outcomes, a hierarchical and domain-based inference strategy will be applied. For the primary outcome domain (i.e., LV function), *p-values* will be adjusted

using Bonferroni correction (two-sided $\alpha=0.05/3=0.0167$). For secondary outcomes, statistical significance will be set at *p-values* <0.01. For biological interpretation, intervention-responsive proteins will be mapped to their corresponding genes, and protein–protein interaction networks and functional enrichment analyses will be explored using the STRING database to identify overrepresented biological pathways and processes, as previously reported.[4,5]

3.2. Adherence and protocol deviations

Adherence will be defined as the percentage of exercise sessions attended by adolescents, recorded by the trainers, divided by the actual number of exercise sessions offered (3 sessions/week during an expected median duration of 5-6 months (expected range duration 14 to 28 weeks). Finally, we will assess the long-term adherence to the intervention (at the 3-month follow-up). This will allow us to evaluate the sustainability of participants' adherence.

3.3. Analysis populations

All primary analyses will be conducted under an intention-to-treat framework, in which all randomized participants will be analyzed in their originally assigned groups regardless of adherence, protocol deviations after randomization, or withdrawal, assuming that missing data are missing at random. Therefore, some adolescents will have valid data at all time points, while others may have missing data at baseline, end of treatment, or the 3-month follow-up.

4. Trial population

4.1. Screening data

Screening data will be based on patients who are defined as eligible by the medical and research team through 4 hospitals located in Madrid city (Spain): *Hospital General Universitario Gregorio Marañón* (HGUGM, #PI20/00645), *Hospital Universitario 12 de Octubre* (HU12OCT, #20/431), *Hospital Universitario Infantil Niño Jesús* (HIUNJ, #R-0028/20), and *Hospital Universitario La Paz* (HULP, #5991). The medical team firstly assesses eligibility of potential participants and engages them in the study. Subsequently, researchers in charge contact participants to interview them and verify compliance with the inclusion and exclusion criteria. Potential participants who meet the criteria are provided with oral and written information about the study.

4.2. Eligibility

We will include both males and females, aged 12-19 years (which is the common age range for patients treated in these units), newly diagnosed with (or having a relapse of) a malignant extracranial tumor, not having received any therapy (except surgery) at diagnosis, showing an adequate health status (Karnofsky $\geq$50, Eastern Cooperative Oncology Group scale score $\leq$2), understanding Spanish or American English, and providing written informed consent. Exclusion criteria are life expectancy <3months and any comorbidity/acute condition contraindicating exercise practice.

4.3. Recruitment

The information necessary for the CONSORT flow diagram will be collected.[6] For the enrolment phase, we will consider the number of patients that are assessed for eligibility by the medical team through the 4 included hospitals and the researchers, the number of excluded patients (plus reason for exclusion), and the number of randomized patients. For the allocation, the number of patients allocated to the intervention, and the number of patients who received or did not receive the intervention (plus reasons) will be noted. For follow-up, the number of patients lost to follow-up and the number who discontinued the intervention (plus reasons) will be considered. Finally, the number of patients included in the analyses using the intention-to-treat principle will be described.

4.4. Withdrawal/follow-up

The number, timing, and reasons (i.e., lost motivation, adverse events, withdrew consent, could not or did not want to attend the post-intervention evaluations) for withdrawal will be counted.

4.5. Baseline patient characteristics

A table will be created to describe the characteristics of the study cohort at baseline. The characteristics include general sociodemographic outcomes (i.e., age, sex, hospital, race), anthropometry (i.e., BMI) and clinical variables (i.e., diagnosis, metastasis, location of metastasis). The characteristics of the total study cohort and each study arm will be summarized using mean (SD) or median (interquartile range) for normally and not normally distributed continuous variables, respectively, and as number (percentage) for categorical variables.

5. Analysis

5.1. Outcome definitions

<u>Primary outcomes</u>

<u>LV function:</u>

1. Change in LV ejection fraction (baseline and 5-6 months). LV ejection fraction will be assessed using color tissue Doppler echocardiography.
2. Change in LV fractional shortening (baseline and 5-6 months). LV fractional shortening will be assessed using color tissue Doppler echocardiography.
3. Change in LV GLS (baseline and 5-6 months). LV GLS will be assessed using 2D-speckle tracking echocardiography.

<u>Secondary outcomes</u>

<u>Cardiac dimensions:</u>

4. Change in LV ejection fraction (baseline and 3-month follow-up). LV ejection fraction will be assessed using color tissue Doppler echocardiography.
5. Change in LV fractional shortening (baseline and 3-month follow-up). LV fractional shortening will be assessed using color tissue Doppler echocardiography.
6. Change in LV GLS (baseline and 3-month follow-up). LV GLS will be assessed using 2D-speckle tracking echocardiography.

7. Change in LV mass (baseline, 5-6 months, and 3-month follow-up). LV mass will be assessed using 2-D guided M-mode imaging.
8. Change in IVS (baseline, 5-6 months, and 3-month follow-up). IVS will be assessed using 2-D guided M-mode imaging.
9. Change in LVEDD (baseline, 5-6 months, and 3-month follow-up). LVEDD will be assessed using 2-D guided M-mode imaging.
10. Change in LVPW (baseline, 5-6 months, and 3-month follow-up). LVPW will be assessed using 2-D guided M-mode imaging.
11. Change in RWT (baseline, 5-6 months, and 3-month follow-up). RWT will be assessed using the following formula: RWT = (IVS + LVPW)/LVEDD.
12. Change in LVH (baseline, 5-6 months, and 3-month follow-up). LVH will be assessed using the age-specific >95th percentile for LV mass indexed by height (in $g \cdot m^{-2.7}$).

Arterial blood pressure:

13. Change in arterial blood pressure (baseline, 5-6 months, and 3-month follow-up). Arterial blood pressure will be assessed using an automatic, cuff-style, upper-arm monitor (Omron M6 Comfort, Omron Global; Kyoto, Japan).

Cardiac damage (blood biomarkers):

14. Change in NT-proBNP (baseline, 5-6 months, and 3-month follow-up). NT-proBNP will be determined with the relevant immunoassay kits on an automated biochemistry analyzer (Cobas C701, Roche Diagnostics; Madrid, Spain).
15. Change in high-sensitivity cardiac troponin-I (baseline, 5-6 months, and 3-month follow-up). High-sensitivity cardiac troponin-I will be determined with the relevant immunoassay kits on an automated biochemistry analyzer (Cobas C701, Roche Diagnostics; Madrid, Spain).

Cardiometabolic-related proteome (blood biomarkers):

16. Change in cardiometabolic-related proteome (baseline, 5-6 months, and 3-month follow-up). Cardiometabolic-related proteome will be assessed using the Olink® Cardiometabolic panel (Olink Target 96 Cardiometabolic Protein assay list), which measures 92 cardiometabolic-related human proteins simultaneously in plasma (expressed in Normalized Protein eXpression (NPX) values, an arbitrary unit presented in Log2 scale) (https://olink.com/products-services/target/cardiometabolic-panel/).

Inflammation (blood biomarkers):

17. Change in high-sensitivity C-reactive protein levels (baseline, 5-6 months, and 3-month follow-up). High-sensitivity C-reactive protein levels will be assessed using a chemistry analyzer (Cobas C701, Roche Diagnostics; Madrid, Spain).

Serum lipid profile and glucose control (blood biomarkers):

18. Change in total cholesterol (baseline, 5-6 months, and 3-month follow-up). Fasting blood samples will be used to assess total cholesterol.
19. Change in high-density lipoprotein cholesterol (baseline, 5-6 months, and 3-month follow-up). Fasting blood samples will be used to assess high-density lipoprotein cholesterol.
20. Change in low-density lipoprotein cholesterol (baseline, 5-6 months, and 3-month follow-up). Fasting blood samples will be used to assess low-density lipoprotein cholesterol.
21. Change in triglycerides (baseline, 5-6 months, and 3-month follow-up). Fasting blood samples will be used to assess triglycerides.
22. Change in apolipoprotein B (baseline, 5-6 months, and 3-month follow-up). Fasting blood samples will be used to assess apolipoprotein B.
23. Change in fasting glycaemia (baseline, 5-6 months, and 3-month follow-up). Fasting blood samples will be used to assess glycaemia.
24. Change in glycated hemoglobin (baseline, 5-6 months, and 3-month follow-up). Fasting blood samples will be used to assess glycated hemoglobin.
25. Change in insulin (baseline, 5-6 months, and 3-month follow-up). Fasting blood samples will be used to assess insulin.
26. Change in homeostasis model assessment-insulin resistance index (baseline, 5-6 months, and 3-month follow-up). Fasting blood samples will be used to assess glucose and insulin, and homeostasis model assessment-insulin resistance index will be computed.

Blood immune phenotype (blood biomarkers):

27. Change in total leukocyte and monocyte count (baseline, 5-6 months, and 3-month follow-up). Total leukocyte and monocyte count will be assessed using a hematology analyzer (Advia 120 Hematology System, Bayer Corporation; Tarrytown, NY).
28. Change in main lymphocyte subpopulations (%) (baseline, 5-6 months, and 3-month follow-up). Main lymphocyte subpopulations will be assessed on fresh blood samples using a multiparametric flow cytometer (FACSCantoTM II, Becton Dickinson and Company BD Biosciences; San Jose, CA) together with BD FACSDiva™ software version 8 (Becton Dickinson and Company BD Biosciences).

Gut microbiome (blood biomarkers):

29. Change in gut microbiome diversity (baseline, 5-6 months, and 3-month follow-up). DNA sequencing to determine gut microbiome diversity (i.e., alpha and beta).
30. Change in specific bacteria abundance (baseline, 5-6 months, and 3-month follow-up). DNA sequencing to determine specific bacteria abundance.

Anthropometry and body composition:

31. Change in body mass index (baseline, 5-6 months, and 3-month follow-up). Body mass index will be calculated dividing body weight in kilograms by the square of the height in meters (kg/m^2).

32. Change in waist-to-hip ratio (baseline, 5-6 months, and 3-month follow-up). Waist-to-hip ratio will be calculated dividing waist by hip circumference (Gulick II Tape Measure, Country Technology, Inc.; Gays Mills, WI) using the same units.
33. Change in lean mass (baseline, 5-6 months, and 3-month follow-up). Lean mass will be assessed using a dual-energy X-ray absorptiometry assessment (Hologic Serie Discovery QDR, Software Physician's Viewer, APEX System Software version 3.1.2.; Bedford, MA).
34. Change in fat mass (baseline, 5-6 months, and 3-month follow-up). Fat mass will be assessed using a dual-energy X-ray absorptiometry assessment (Hologic Serie Discovery QDR, Software Physician's Viewer, APEX System Software version 3.1.2.; Bedford, MA).
35. Change in visceral fat (baseline, 5-6 months, and 3-month follow-up). Visceral fat will be assessed using a dual-energy X-ray absorptiometry assessment (Hologic Serie Discovery QDR, Software Physician's Viewer, APEX System Software version 3.1.2.; Bedford, MA).
36. Change in bone mineral density of the total body (less head) (baseline, 5-6 months, and 3-month follow-up). Bone mineral density of the total body (less head) will be assessed using a dual-energy X-ray absorptiometry assessment (Hologic Serie Discovery QDR, Software Physician's Viewer, APEX System Software version 3.1.2.; Bedford, MA).
37. Change in bone mineral density of the femoral neck (baseline, 5-6 months, and 3-month follow-up). Bone mineral density of the femoral neck will be assessed using a dual-energy X-ray absorptiometry assessment (Hologic Serie Discovery QDR, Software Physician's Viewer, APEX System Software version 3.1.2.; Bedford, MA).

Energy intake:

38. Change in energy intake (baseline, 5-6 months, and 3-month follow-up). Energy intake will be estimated using the Nutrimind software.
39. Change in substrate intake (baseline, 5-6 months, and 3-month follow-up). Substrate intake will be estimated using the Nutrimind software.

Physical activity:

40. Change in physical activity (baseline, 5-6 months, and 3-month follow-up). Physical activity will be assessed using the 'Youth Activity Profile' (YAP) and 'Assessment of Physical Activity Levels' (APAL) questionnaires.
41. Change in sedentary behaviors (baseline, 5-6 months, and 3-month follow-up). Sedentary behaviors activity will be assessed using the 'Youth Activity Profile' (YAP) and 'Assessment of Physical Activity Levels' (APAL) questionnaires.

Cardiopulmonary exercise testing:

42. Change in VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$) at peak (baseline, 5-6 months, and 3-month follow-up). VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$) at peak will be assessed using a ramp-like bicycle ergometer.
43. Change in VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$) at the ventilatory threshold (baseline, 5-6 months, and 3-month follow-up). VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$) at the ventilatory threshold will be assessed using a ramp-like bicycle ergometer.

44. Change in power output (watts) at peak (baseline, 5-6 months, and 3-month follow-up). Power output (watts) at peak will be assessed using a ramp-like bicycle ergometer.
45. Change in power output (watts) at the ventilatory threshold (baseline, 5-6 months, and 3-month follow-up). Power output (watts) at the ventilatory threshold will be assessed using a ramp-like bicycle ergometer.

Muscle strength:

46. Change in dynamic muscle strength (baseline, 5-6 months, and 3-month follow-up). Dynamic muscle strength will be assessed using specific stationary weight training machines performing 5 repetitions until momentary muscular exhaustion for leg and bench press exercise, as well as for seated lateral row, lateral pull down and knee extension.
47. Change in handgrip strength (baseline, 5-6 months, and 3-month follow-up). Handgrip strength will be assessed using a handheld digital Smedley dynamometer (TKK 5401, Takei Scientific Instruments Co., Ltd., Niigata, Japan).
48. Change in maximum inspiratory muscle strength (baseline, 5-6 months, and 3-month follow-up). Maximum inspiratory muscle strength will be assessed using a mouth pressure meter (CareFusion MicroRPM Respiratory Pressure Meter; Kent, UK).

Functional mobility:

49. Change in functional mobility (baseline, 5-6 months, and 3-month follow-up). Functional mobility will be assessed using the Timed Up and Go, Timed Up and Down Stairs, and 30-second chair stand tests.
50. Change in range of motion of the ankle (baseline, 5-6 months, and 3-month follow-up). Range of motion of the ankle will be assessed using a goniometer (Baseline Evaluation Instruments, Fabrication Enterprises Inc.; Elmsford, NY).

Psychological status:

51. Change in health-related quality of life (baseline, 5-6 months, and 3-month follow-up). Health-related quality of life will be assessed using the Pediatric Quality of Life Inventory (PedsQL) 3.0 (Patients and Tutor's version, Cancer Module).
52. Change in cancer-related fatigue (baseline, 5-6 months, and 3-month follow-up). Cancer-related fatigue will be assessed using the Pediatric Quality of Life Inventory (PedsQL) 3.0 (Patients and Tutor's version, Multidimensional Fatigue Scale).

Clinical variables:

53. Survival (baseline, and 3-month follow-up). Survival will be assessed from diagnosis to the end of the study or death using medical records.
54. Treatment tolerability (baseline, and 5-6 months). Treatment tolerability will be assessed as the number of days of treatment interruption/delay and hospitalization length (additional/prolonged hospitalization during treatment) using medical records.

55. Change in the number and duration of viral/bacterial/fungal infections (baseline, 5-6 months, and 3-month follow-up). Number and duration of viral/bacterial/fungal infections will be retrieved from medical records.
56. Change in the number of treatment-related side effects (or 'events') (baseline, 5-6 months, and 3-month follow-up). Treatment-related side effects (or 'events') will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.
57. Change in toxicity grade (baseline, 5-6 months, and 3-month follow-up). Toxicity grade will be assessed using the formula from Langlais et al (2022).

Adherence:

58. Adherence to the exercise intervention (baseline and 5-6 months). Adherence will be assessed using the percentage of exercise sessions attended and divided by the actual number of exercise sessions offered.

5.2. Analysis methods

The effects of the exercise intervention on primary and secondary outcomes will be assessed separately using constrained baseline (meaning baseline adjusted) linear mixed models (cLMMs) with random intercepts for participants. Repeated measurements, including baseline and follow-up assessments, will be modeled as a function of evaluation time, randomly assigned group, and their interaction, with group means constrained to be equal at baseline.[7] Intervention effects will be estimated from the group-by-time interaction contrasts at post-intervention. In addition, pre-specified adjusted models will include age, sex, and tumor type according to the International Classification of Childhood Cancer, third edition (ICC3),[8] as covariates. Categorical outcomes will be compared between groups using chi-square or Fisher's exact test, as appropriate based on expected cell counts.

To address multiplicity due to the large number of outcomes, a hierarchical and domain-based inference strategy will be applied. For the primary outcome domain (*i.e.*, LV function), *p-values* will be adjusted using Bonferroni correction (two-sided $\alpha=.05/3=.0167$). For secondary outcomes, statistical significance will be set at *p-values* <0.01. For biological interpretation of proteome data, intervention-responsive proteins (*i.e.*, differentially expressed) will be mapped to their corresponding genes, and protein–protein interaction networks and functional enrichment analyses will be explored using the STRING database to identify overrepresented biological pathways and processes, as previously reported.[4,5] Overall survival will be described using Kaplan–Meier curves, defined as time from randomization to death from any cause, with censoring at the end of follow-up. Given the expected low number of events, survival analyses will be considered exploratory and primarily descriptive, and any between-group comparisons will be interpreted with caution.

All primary analyses will be conducted under an intention-to-treat framework, in which all randomized participants will be analyzed in their originally assigned groups regardless of adherence, protocol deviations after randomization, or withdrawal, assuming that missing data are missing at random. No pre-specified interim analyses will be performed and hence, a stopping guidance will not be applicable.

Strict access controls will be put in place to ensure that data are securely stored on hospital computer systems, preserving confidentiality and data integrity. To maintain high data quality, weekly quality assurance reviews will be performed to detect potential issues, including missing data or forms, values outside predefined ranges, incorrect data entries, implausible or inconsistent dates over time, discrepancies across study forms and visits, and incomplete fields without justified reasons. The main statistical analyses will be performed by an independent researcher who is not involved in the recruitment, evaluations, and interventions, and will be conducted blinded to the treatment allocation by coding the intervention arms (i.e., A, B).

5.3. Missing data

We will report and explore missing data and possible patterns. We expect that missing data will be assumed as missing at random. Therefore, the linear mixed model analyses will handle the missing data. Nevertheless, we will reconsider this assumption once the data processing is finalized. If we believe this assumption does not hold, we will take appropriate measures for the data analyses by using other multiple imputation techniques, for example.

5.4. Additional analyses

Changes in other outcomes will be analyzed using a similar protocol as described by '5.2 Analysis methods' unless other analyses would be more appropriate depending on the outcome.

5.5. Harms

Ascertained adverse events related to exercise testing or training sessions, including muscle pain, fatigue, and general aches and pains according to patients/parents/caregivers (self-reported) will be collected by each study arm. No formal statistical testing will be undertaken.

5.6. Statistical software

The analyses will be performed using R. For the main analyses we will use the 'lme4' or 'LMMstar' package. The use of packages will be reported in the manuscript.

6. References

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